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TECHNICAL REPORT

NATICK/TR-82/006

2, 4, 6-TRINITROTOLUENE - SURFACTANT COMPLEXES, BIODEGRADABILITY, MUTAGENICITY AND SOIL LEACHING STUDIES

by

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and

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January 1982

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT LABORATORIES
NATICK, MASSACHUSETTS 01760

Science and Advanced Technology Laboratory

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NATICK/TR-82/006	2. GOVT ACCESSION NO. AD-A113 727	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) 2,4,6-TRINITROTOLUENE - SURFACTANT COMPLEXES, BIODEGRADABILITY, MUTAGENICITY AND SOIL LEACHING STUDIES		5. TYPE OF REPORT & PERIOD COVERED Final Report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) D. L. Kaplan and A. M. Kaplan		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Natick Research & Development Labs. Science & Advanced Technology Laboratory, DRDNA-YEP, Natick, MA 01760		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Program Element 6.2 Project 1L162720D048 Work Unit 02-040/R915.03.1342
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Toxic & Hazardous Materials Agency Aberdeen Proving Ground, MD 21010		12. REPORT DATE Sep. 81 13. NUMBER OF PAGES 48
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release, distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

TRINITROTOLUENE	AMINO SURFACTANTS	LEACHING
TNT	ENVIRONMENTAL HAZARDS	PHOTOLYSIS
CONTAMINATION	SOILS	
WASTEWATER	SEDIMENTS	
BIODEGRADATION	MUTAGENS	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Amino surfactants at alkaline pHs precipitate 2,4,6-trinitrotoluene (TNT) as a water insoluble complex. Mutagenicity studies indicated these complexes were more potent mutagens than TNT. Soil leaching studies demonstrated that in situ immobilization of TNT in contaminated soils would not be advisable due to the large quantities of surfactant required, the leaching of excess surfactant and sodium hydroxide organic matter extracts into the groundwater, and the inability of the surfactant treatment to immobilize TNT microbial reduction

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products. Biodegradation studies in soil and water showed there are potential problems with the long term stability of these complexes. Water soluble materials were produced nonbiologically. These compounds were not TNT or any of seven TNT reduction products. Upon concentration, mutagenic activity was found with the filtrate after treatment and filtration of Composition B waste water with Duoquad T-50 at pH 11 in a pilot scale facility.

PREFACE

Significant environmental hazards result from 2,4,6-trinitrotoluene (TNT) contamination of waste waters and soils. Many alternative treatments have been proposed to remove TNT. Recently the use of amino surfactants under alkaline conditions has been considered to precipitate TNT as a water-insoluble complex and to stabilize TNT in contaminated soils. This report describes studies on the biodegradability of these TNT-surfactant complexes in soil and water, and their mutagenic properties. The potential of this method for the *in situ* treatment of TNT contaminated soils has been evaluated.

This work was supported by the U.S. Army Toxic and Hazardous Materials Agency under project numbers P112.03.03 and R915.03.1342.

We wish to thank Dr. Y. Okamoto of the Polytechnic Institute of New York for his helpful conversations and a supply of surfactants, and Drs. L.D. Metcalf and S.H. Shapiro, Armak Corp., McCook, IL, for information about the surfactants. We also thank Mr. Carmine DiPietro of the Science and Advanced Technology Laboratory for his GC/MS analysis and Ms. P. Riley, Ms. P. Lapinskas and Mr. S. Cowburn of the Science and Advanced Technology Laboratory for their technical assistance.

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INTRODUCTION

Environmental hazards result from 2,4,6-trinitrotoluene (TNT) contamination of waste waters, soils, and sediments from the operation of munitions manufacturing, loading, assembling, and packing facilities. TNT has been identified in the ground water after leaching from disposal sites.[1]

TNT has been shown to cause liver damage and anemia in humans,[2] and is toxic to rats, mice,[3] fish,[4,5] unicellular green algae, copepods and oyster larvae.[6] TNT also inhibits the growth of many fungi, yeasts, actionmycetes,

[1]W. E. Pereira, D. L. Short, D. B. Manigold and P. K. Roscio. 1979. Isolation and Characterization of TNT and its Metabolites in Groundwater by Gas Chromatograph-Mass Spectrometer-Computer Techniques. Bull. Environm. Contam. Toxicol. 21: 554-562.

[2]N. I. Sax. 1963. Dangerous Properties of Industrial Materials, 2nd Ed. Reinhold Publishing Corp., NY.

[3]C. C. Lee, J. V. Dilley, J. R. Hodgson, D. O. Helton, W. J. Wiegand, D. N. Roberts, B. S. Anderson, L. M. Halfpap, L. D. Kurtz and N. West. 1975. Mammalian Toxicity of Munition Compounds: Acute Oral Toxicity, Primary Skin Irritation, Dermal Sensitization and Disposition and Metabolism. Midwest Research Inst., Kansas City, MO. 101 pp.

[4]J. L. Osmon, and R. E. Klausmeier. 1972. The Microbial Degradation of Explosives. Dev. Ind. Microbiol. 14: 247-252.

[5]M. W. Nay, C. W. Randal and P. H. King. 1974. Biological Treatability of Trinitrotoluene Manufacturing Wastewater. J. Water Pollut. Control Fed. 46: 485-497.

[6]W. D. Won, L. H. DiSalvo and J. Ng. 1976. Toxicity and Mutagenicity of 2,4,6-Trinitrotoluene and its Microbial Metabolites. Appl. Environ. Microbiol. 31: 576-580.

and Gram-positive bacteria,[7] and is mutagenic in the Ames test.[8]

The body of literature concerning the biological fate of TNT in mammalian and microbial systems agrees that the aromatic nucleus of the TNT molecule is not cleaved but undergoes transformations. These transformations are well documented, occurring under aerobic and anaerobic conditions, and include sequential reduction of nitro groups through a hydroxylamino intermediate to the amino analog, or formation of the corresponding azoxy compounds.[9,10,11,12,13] In mammals, glucuronide conjugates have been identified,[14] and in microbiological systems, insoluble macromolecules have been characterized.[15]

[7]R. E. Klausmeier, J. L. Osmon and D. R. Walls. 1973. The Effect of Trinitrotoluene on Microorganisms. Dev. Ind. Microbiol. 15: 309-317.

[8]J. V. Dilley, C. A. Tyson and G. W. Newell. 1979. Mammalian Toxicological Evaluation of TNT Wastewaters. V. III. Acute and subacute Mammalian Toxicity Condensate Water. SRI International, Menlo Park, CA.

[9]H. H. Dale. 1921. The Fate of TNT in the Animal Body. Spec. Rep. Ser. Med. Res. Coun. (G. B.) 58: 53-61.

[10]R. Lemberg and J. P. Callahan. 1944. Metabolism of Symmetrical Trinitrotoluene. Nature (London) 154: 768-769.

[11]N. G. McCormick, F. E. Feeherry and H. S. Levinson. 1976. Microbial Transformation of 2,4,6-Trinitrotoluene and Other Nitroaromatic Compounds. Appl. Environ. Microbiol. 31: 949-958.

[12]R. P. Naumova, N. N. Amerkhanova and V. A. Shaykhutdinov. 1979. Study of The First Stage of Trinitrotoluene Transformation by *Pseudomonas Denitrificans*. Prikladnaya Biochem. Microbiol. 15: 45-50.

[13]See reference 1, p. 7.

[14]H. J. Channon, G. T. Mills and R. T. Williams. 1944. The Metabolism of 2,4,6-Trinitrotoluene (α-T.N.T.). Biochem. J. 38: 70-85.

[15]D. F. Carpenter, N. G. McCormick, J. H. Cornell and A. M. Kaplan. 1978. Microbial Transformation of ^{14}C-labelled 2,4,6-Trinitrotoluene in an Activated-Sludge System. Appl. Environ. Microbiol. 35: 949-954.

The reduction products arising from the metabolism of TNT also pose a significant environmental hazard since they are toxic to rats and mice[16] and mutagenic in the Ames test.[17]

The problems associated with the discharge of TNT into the environment have prompted investigations into a number of waste treatment alternatives, including carbon absorption, lagoon storage, photolysis and chemical treatments. Recent findings indicate certain amine surfactants under alkaline conditions rapidly complex TNT to form a water-insoluble, nonexplosive precipitate which can be separated from the effluent and incinerated or land-filled.[18,19,20] An on-line process for the purification of wastewater contaminated with TNT, based on this reaction appeared feasible since only traces of TNT were found after treatment and filtering. The process has also been proposed for *in situ* treatment of contaminated lagoons and soils.

The same surfactant treatment also may be used with other munition wastes. Under these conditions the hydrolysis of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and nitroglycerine

[16]H. V. Ellis, J. R. Hodgson, S. W. Hwang, L. M. Halfpap, D. O. Helton, B. S. Anderson, D. L. VanGoethem and C. C. Lee. 1978. Mammalian Toxicity of Munitions Compounds Phase I: Acute Irritation, Dermal Sensitization, Disposition and Metabolism, and Ames Tests of Additional Compounds. Project No. 3900-B, Midwest Res. Inst., Kansas City, MO. 39 pp.

[17]See reference 8, p. 8.

[18]Y. Okamoto and J. Y. Wang. 1977. Micellar Effects on the Reaction of 2,4,6-Trinitrotoluene with Amines. J. Org. Chem. 42: 1261-1262.

[19]M. Croce and Y. Okamoto. 1978. Cationic Micellar Catalysis of the Aqueous Alkaline Hydrolyses of 1,3,5-Triaza-1,3,5-Trinitrocyclohexane and 1,3,5,7-Tetraaza-1,3,5,7-Tetranitrocyclooctane. J. Org. Chem. 44: 2100-2103.

[20]Y. Okamoto, J. Y. Wang and E. J. Chou. 1978. Removal of Trinitrotoluene from Aqueous Media. U.S. Patent No. 4,073,726.

is accelerated.[21] In these reactions the surfactant serves as a catalyst for the hydrolysis under alkaline conditions. An insoluble complex is formed with 2,4-dinitrotoluene.

Many of the amino surfactants utilized to precipitate TNT are used as biocides. However, in low concentrations they are biodegradable under certain conditions.[22,23]

It is the purpose of this work to evaluate the biodegradability of these TNT-surfactant complexes in soil and water and to assess their mutagenicity. A further objective is to determine the effectiveness of a proposed treatment of TNT-contaminated soils that involves complexing with surfactants to immobilize the TNT and prevent further leaching into the groundwater.

MATERIALS AND METHODS

Chemicals: Commercially available TNT (Eastman Kodak, Rochester, NY) was recrystallized. Dr. J. C. Hoffsommer, U.S. Naval Surface Weapons Center, Silver Spring, MD, supplied samples of 2-amino-4,6-dinitrotoluene (2A), 4-amino-2,6-dinitrotoluene (4A), 2,4-diamino-6-nitrotoluene (2,4DA), 2,6-diamino-4-nitrotoluene (2,6DA), 4-hydroxylamino-2,6-dinitrotoluene (4OHA), 2,2',6,6'-tetranitro-4,4'-azoxytoluene (4,4'Az) and 4,4',6,6'-tetranitro-2,2'-azoxytoluene (2,2'Az).

[21]See reference 19, p. 9.

[22]H. J. Hueck, D. M. M. Adema and J. R. Wiegmann. 1966. Bacteriostatic, Fungistatic and Algistatic Activity of Fatty Nitrogen Compounds. Appl. Microbiol. 14: 308-319.

[23]L. J. Gawel and R. L. Huddleston. 1972. The Biodegradability of Low Concentrations of Certain Quarternary Ammonium Antimicrobials by Bacteria. Am. Oil Chem. Soc. Meeting. Los Angeles, CA.

The surfactants Duoquad T-50 (N-tallow-N'N'N'-trimethyl-N,N-dimethyl-1,3-diamino-propane, 50% active solution), Duomeen T (N-tallow-1,3-diamino-propane, white waxy solid) and Arquad T-50 (N-tallow-trimethylammonium surfactant, 50% active solution) were supplied by Dr. Y Okamoto, Polytechnic Institute of NY, and are commercially available from Armak Corp., McCook, IL. Dr. Okamoto also supplied non-radioactive (cold) TNT-surfactant complexes.

Synthesis of Labelled Complexes: ^{14}C-TNT, 4.18 x 10^5 Bq (11.3 μCi) per mg and uniformly ring labelled, was synthesized from ^{14}C-toluene (New England Nuclear, Boston, MA). The ^{14}C-labelled complexes were synthesized by reacting individual surfactants with the ^{14}C-labelled TNT and cold TNT at molar ratios of 0.4, 3.0 and 3.0 surfactant to TNT with Duoquad T-50, Arquad T-50, and Duomeen T (Figure 1). The surfactant was first mixed with the 140 ppm (μg/ml) TNT solution, and

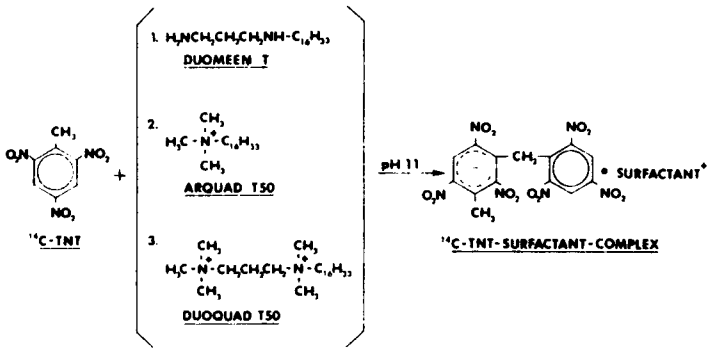

Figure 1. Synthesis of radioactively labelled TNT-surfactant complexes.

then the pH was adjusted to 11.0 with 1N NaOH at 40°C. The insoluble precipitate began forming immediately and the reaction was allowed to proceed for 5 hours. The dark brown precipitates were filtered and washed continuously with warm water. Aliquots of the washes were counted in a Parkard TriCarb Scintillation Counter Model 3255 until no counts above background were detected. The precipitates were resolubilized with acetone in the case of Duoquad T-50 (96.2 Bq or 0.0026 μCi per mg) and methylene chloride with Duomeen T (2.55×10^2 Bq or 0.0069 μCi per mg) and Arquad T-50 (4.44×10^2 Bq or 0.012 μCi per mg).

Soil Columns: Soil columns, 25 cm high by 21 cm diam., were filled with 1.2 kg (dry wt) of a Hinckley soil which had been passed through a 2-mm sieve (Figure 2).

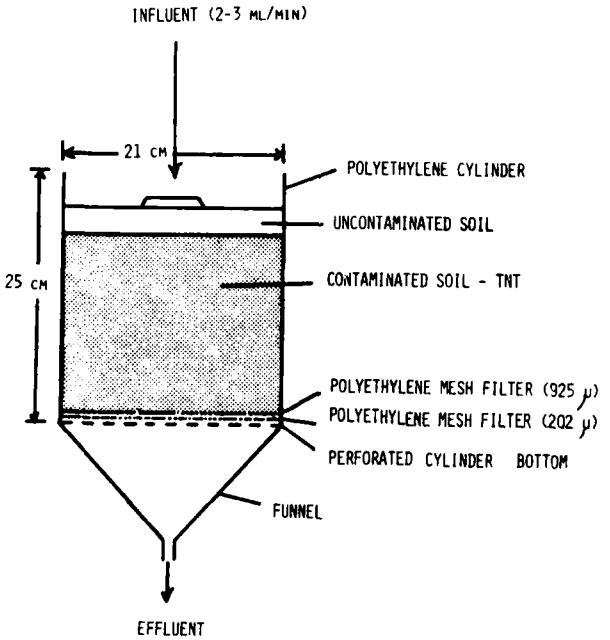

Figure 2. Diagram of soil column design.

This fast draining soil was chosen as a worst-case situation. The bottom of the columns were fitted with two polyethylene screens, 925 μm and 202 μm pore sizes. The soil had a pH of 5.5 and 1.34% organic matter by ignition. TNT, 600 mg per column, for a 50-ppm concentration, was dissolved in acetone and then added to each column. The acetone was allowed to evaporate and then the TNT was mixed into the top 2.5 cm of soil which in turn was overlaid with 4 cm of uncontaminated soil.

Soil Column Treatments: Influent solutions, 300 mL per treatment day, were dripped onto the columns at 2 to 3 mL per minute. Effluents were collected, filtered through a 0.2-μm filter unit and analyzed directly. Initially, all four columns received the same treatment, 300 mL distilled water per day for the first 35 treatment days, to allow for column stabilization. On day 36 the four different treatments were initiated. Column A continued to receive 300 mL of distilled water per treatment day, column B received 300 mL distilled water adjusted to pH 11.0 ± 0.5 with 5N NaOH, column C received 0.1 M Duoquad T-50 surfactant in 300 mL distilled water, and column D received 300 mL distilled water containing 0.1 M Duoquad T-50, and the solution was adjusted to pH 11.0 ± 0.5 with 5N NaOH.

Analysis of Column Effluents: Effluents from TNT contaminated soil columns were analyzed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography/mass spectroscopy (GC/MS). TLC was performed on silica gel LK5DF plates (Whatman, Clifton, NJ). The solvent systems were chloroform/methanol/acetic acid (90/10/1) and benzene/hexane/acetone (50/50/1.5). Spots were visualized with ultraviolet light and by spraying with tetramethylammonium hydroxide.

HPLC was performed on a Waters Liquid Chromatograph equipped with two Model 6000A solvent pumps, Model 450 variable wavelength detector set at 230 nm Data Module and Model 720 System Controller. The mobile phase was methanol/water (50/50, v/v). Injections were 50 µL onto a 30-cm by 3.9-mm I.D. Waters µBondapak reverse phase column at room temperature. The flow rate was 2.5 mL per minute. Samples were analyzed directly, but when concentrations fell below detection limits, effluent samples were concentrated by drawing them through C-18 SEP-PAK cartridges with µBondapak packing (Waters Assoc., Milford, MA). The cartridges were eluted with a small volume of methanol which was analyzed directly.

Peaks of interest on liquid chromatograms were collected, concentrated and analyzed by GC/MS. Extracts of column effluents were also prepared by continuous extraction of 250 mL volumes with ether for 24 hours. GC/MS analyses were performed on a Finnigan Model 4000 system. Standards and unknowns were chromatographed with temperature programming on a 15-mm by 0.22-mm ID SE54 FS glass capillary column. Injection and transport lines were 200°C. The helium flow was 80 cm per second and injection volumes were 1 µL. The column temperature was programmed for 1 minute at 150°C, 150°C to 200°C at 10°C per minute, and 200°C for 5 minutes.

Determination of Duoquad T50: Duoquad T50 concentrations in soil column effluents were determined by TLC on cellulose plates, without fluorescent indicator in n-butanol/ethyl acetate/water (2/1/1). Developing time was 1.5 hours and the $R_f \tilde{=} 0.47$. Plates were visualized with iodine vapors and 1 µg was detectable. At high concentrations a second spot appeared at $R_f = 0.78$ and indicated an impurity. The R_f varied with concentration; therefore, a range of standard concentrations was run with the unknowns.

Standard Leaching Test: To further evaluate the soil columns after completion of the long-term leaching tests, soil samples from each of the four columns were subjected to a standard leaching test to determine the maximum concentration of compounds present.[24] Samples were rotated 24 hours for each extraction step and there were three washes for each column.

Aqueous Biodegradation Studies: The ^{14}C-labelled complexes (1.66×10^3 Bq or 0.045 μCi) were added to the liquid media along with unlabelled complex to a total concentration of 0.1%. The complexes were dissolved in solvent (acetone or methylene chloride) and added to 20 mL of distilled water or 4 g/L of nutrient broth (Difco). The solvents were evaporated on a hot plate for 10 minutes and the total volume of media brought up to 100 mL for anaerobic incubations in 125-mL Erlenmeyer flasks. The flasks were adapted with ground glass joints fitted with a gas outlet tube, fitted directly to scintillation vials containing 1 mL of 1N NaOH. The vials were changed twice weekly during the first three months of incubation, and weekly during the remaining period. Static anaerobic studies were incubated at 37°C without aeration. Controls consisted of distilled water or nutrient broth without the complexes, and flasks containing distilled water with three different ^{14}C-labelled complexes incubated under the same conditions. These distilled water controls were run in duplicate while the nutrient broth incubations were run in triplicate.

The aerobic incubation of ^{14}C-labelled TNT-Duoquad-complex consisted of 2 trains of traps, one for the control (complex in distilled water), and one for the nutrient broth (4 g/L) incubation with the complex. The incubations

[24] R. K. Ham, M. A. Anderson, R. Stegman and R. Stanforth. 1979. Comparison of Three Waste Leaching Tests. EPA 600/2-79-071.

consisted of 100 mL of media in 250-mL flasks Erlenmeyer flasks and the complex was added as described previously. Compressed air flowed at 100 mL per minute through a 5N sodium hydroxide trap; a 1N HCl trap; an empty trap to prevent carryover; the sample, a second empty trap; and a 1N NaOH trap to collect $^{14}CO_2$. A second train was used for the control. The traps were changed weekly and the ^{14}C counted as before. Aerobic incubations were at room temperature.

Aerobic cultures were inoculated with activated sludge from the Marlborough Easterly sewage treatment plant (Marlborough, MA) and anaerobic cultures with digest from the Nut Island sewage treatment plant (Boston, MA). The aerobic and anaerobic sludges contained <0.1% and 1.7% total solids. Sludge samples were diluted 100-fold with 0.85% KCl, filtered, and 0.5 mL was added to the culture flasks.

Soil Biodegradation Studies: Soil incubations were run with 30 g (dry wt) of garden soil containing 5.9% organic matter by ignition and a pH of 6.4. Incubations were conducted in 125-mL Erlenmeyer flasks as described for the aqueous incubations. Anaerobic (unaerated) incubations contained the three complexes individually in sterile and unsterilized soil. Samples were sterilized by two different methods, steam autoclaving for two 45-minute runs on consecutive days, or by exposure to ethylene oxide gas for 24 hours.[25,26] To examine the effects of sterilization on the complexes themselves, they were added to the flasks both before or after sterilization. For sterile control flasks, the complexes in solvent were added to 5 g of oven dry soil with 2 mL distilled water; the solvent was evaporated in an oven at 60°C and the remaining 25 g (dry wt) of

[25]L. E. Allison. 1951. Vapor-Phase Sterilization of Soil with Ethylene Oxide. Soil Sci. 72: 341-351.

[26]K. Kereluk and R. S. Lloyd. 1969. Ethylene Oxide Sterilization. J. Hospital Res. 7: 30.

fresh undried soil was added and mixed. These flasks were then autoclaved or gas-sterilized. For nonsterile flasks with complexes which had undergone sterilization, the complexes were added to 5 g of oven dry soil with 2 mL of distilled water, the solvent evaporated, and the flasks sterilized. Then, 25 g of fresh undried soil was added and mixed. For nonsterilized soil flasks with nonsterilized complexes, the complex was added to 5 g of oven dry soil with 2 mL distilled water, the solvent evaporated, and 25 g of fresh undried garden soil was added. Apart from the sterile controls, soil without any complex added also served as a background control.

Aerobic incubations were conducted with the ^{14}C-TNT-Duoquad T-50 complex in two trains of gas traps as described previously. The control was soil-sterilized by autoclaving with the complex added after. Aerobic incubations were conducted in 250-mL Erlenmeyer flasks at room temperature. Anaerobic control incubations were conducted in both soil and water with ^{14}C-UL-sucrose as substrate to assure efficient operation of the $^{14}CO_2$ trapping systems and to evaluate the efficiency of gas and steam sterilization procedures in the soil studies.

Fraction Scheme for Aqueous Studies: After 221 days (anaerobic) and 202 days (aerobic) the media from the aqueous incubations were acidified with 0.5 mL of 6N HCL to evolve residual $^{14}CO_2$ which was trapped. The liquid was then centrifuged at 7,000 rpm for 15 minutes and passed through a 0.2-μm filter. The cell pellet was washed with 0.85% KCl and centrifuged. The pellet and filter pad were counted for radioactivity. The filtrate was passed through a C-18 SEP-PAK cartridge. Aliquots of influent and effluent from the cartridge were counted for radioactivity. The cartridge was eluted with methanol, which was divided into three aliquots for HPLC, TLC, and scintillation counting.

The empty flasks which originally contained the media, were rinsed with 15 mL of acetone or methylene chloride and then with 0.5 mL dimethylsulfoxide. The solvent extracts were counted for radioactivity and the first solvent wash was analyzed by HPLC and TLC (Figure 3).

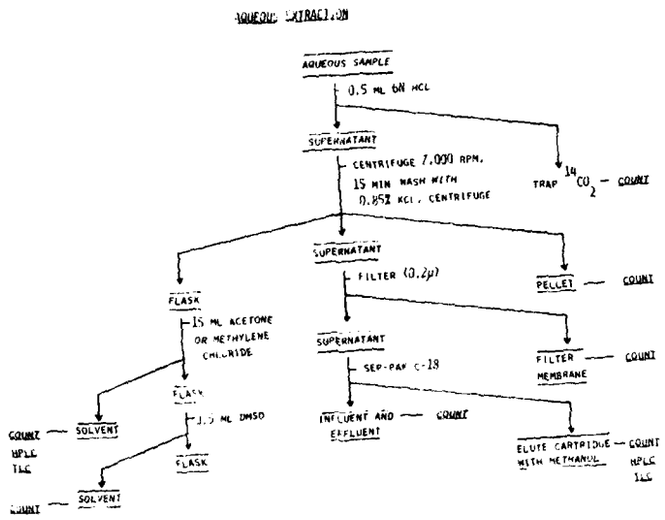

Figure 3. Extraction scheme for aqueous incubations.

Fractionation Scheme for Soil Studies: After 223 days (anaerobic) and 209 days (aerobic) the soil samples were oven-dried at 45°C for 24 hours. The samples were then extracted for 24 hours with acetone in a Soxhlet extractor followed by a 24-hour Soxhlet-extraction with methylene chloride. The solvent

extracts were counted for radioactivity and examined by HPLC and TLC. The soil samples were redried overnight at 100°C and then incinerated at 800°C to 900°C to trap any bound (not solvent extractable) ^{14}C-material as $^{14}CO_2$. The flasks originally containing the soil samples were solvent washed and analyzed in the same manner as in the aqueous extraction procedure (Figure 4).

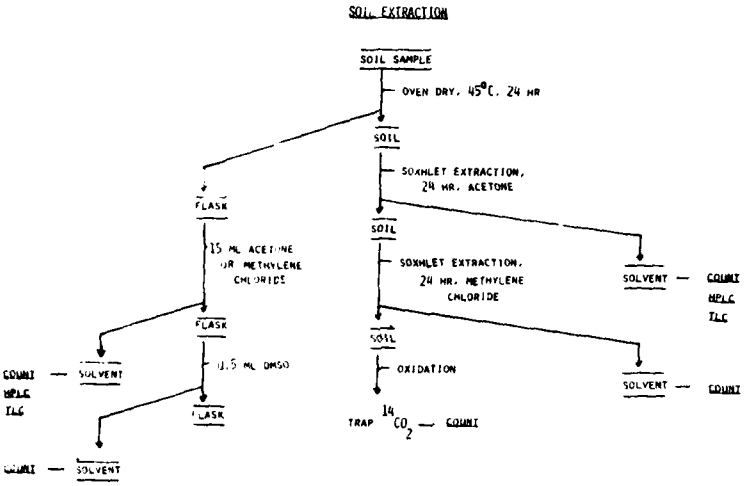

Figure 4. Extraction scheme for soil incubations.

Incineration of Soil Samples: After extraction of the incubated soil samples containing the ^{14}C-TNT-surfactant complexes, the entire 30-g soil sample was transferred to a quartz tube (1.3 cm ID by 33 cm long) which was swept with oxygen. The sample was incinerated in a Lindberg oven at 800°C to 900°C for a total of two hours.

The end of the tube within the oven was filled with cupric oxide. Effluent gases passed through a 6N HCl trap and then through 100 ml of 5N NaOH to ensure entrapment of all the $^{14}CO_2$. An aliquot of this solution was assayed for radioactivity.

Analysis of Reduction Products: The extracts from the aqueous and soil incubations were analyzed for decomposition products by HPLC, TLC, and scintillation counting. HPLC was performed on a Waters HPLC system as described earlier. Eight potential metabolites were determined on a methanol/water programed gradient which was initiated at 40% methanol and terminated at 78% methanol over a run time of 15 minutes (Figure 5). Detection was at 230 nm and the flow was 2.5 mL per minute through a μBondapak reverse phase column. Injection

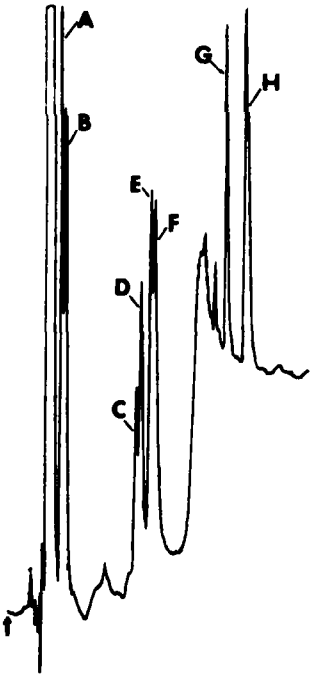

Figure 5. Solvent program run illustrating the separation of TNT and seven reduction products by HPLC (0.1 AUFS). A=2,6DA; B=2,4DA; C=4OHA; D=TNT; E=4A; F=2A; G=2,2'Az; H=4,4'Az.

volumes varied up to 200 L. Retention times in minutes were: 2.19, 2.35, 5.60, 5.84, 6.40, 6.63, 10.89, and 11.85 for 2,6DA, 2,4DA, 4OHA, TNT, 4A, 2A, 2,2'Az, and 4,4'Az, respectively. Concentrations down to 100 ppb (ng/mL) were routinely detected by direct injection. Using SEP-PAK cartridges, concentrations near 1 ppb could be detected with the exception of 2,6DA and 2,4DA.[27]

TLC was performed on silica gel LK5DF plates in the solvent systems described earlier. Visualization was by UV light or by spraying with tetramethylammonium hydroxide. All eight standards were run concurrently with each plate. The spots were visualized, and then the plates were scored in one-tenth increments. The media in each increment were scraped into scintillation vials and counted. In benzene/hexane/acetone, the R_f values and colorations were 0.29 (purple), 0.44 (purple) and 0.70 (rust) for 2,2'Az, 4,4'Az, and TNT. In chloroform/methanol/acetic acid, they were 0.68 (yellow), 0.67 (yellow), 0.61 (purple) and 0.56 (yellow) for 2,4DA, 2A and 4A, 4OHA and 2,6DA.

Mutagenicity Testing: The Ames screening test for mutagenicity was performed according to standard procedures.[28,29] The surfactants (Duoquad T-50, Arquad T-50, and Duomeen T), TNT, and the three TNT-surfactant complexes were tested. Five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538), were used to test these compounds over a range of concentrations with and without metabolic activation. The tests were run in triplicate and a three-

[27]D. L. Kaplan and A. M. Kaplan. 1981. Analytical Method for Concentration of Trace Organics from Water. NATICK/TR-81/014. US Army Natick Research and Development Laboratories, Natick, MA.

[28]B. N. Ames. 1979. Supplement to the Methods Paper. Univ. Calif. Berkeley.

[29]B. N. Ames, J. McCann, and E. Yamasaki. 1975. Methods for Detection Carcinogens and Mutagens with the *Salmonella* Mammalian-Microsome Mutagenicity Test. Mut. Res. 31: 347-364.

fold increase in back mutations was considered as the criterion for a positive test for mutagenicity.

A sample of filtrate from a Composition B (40% TNT, 60% RDX) waste stream, treated with Duoquad T-50 at pH 11 and filtered in a pilot scale facility, was also tested for mutagenicity. This test was conducted to evaluate the quality of the filtrate after process treatment and filtration. The filtrate, adjusted to pH 7.0, was diluted and concentrated in order to evaluate the response over a range of concentrations. Concentration was accomplished by rotary evaporation at 45°C. The solution changed from yellow to orange upon concentration.

The Duomeen T-50-TNT complex was insoluble in dimethylsulfoxide. Formamide, dimethylformamide, 1-methyl-2-pyrrolidinone, tetrahydrofurfuryl alcohol, tetrahydrofuran, and acetonitrile were alternative solvents also rejected for use in the Ames test on the basis of either low solubility of the Duomeen complex or toxicity or mutagenicity of the solvent to *S. typhimurium*. Methylene chloride was selected as the solvent despite its mutagenic effect on TA98 because the complex is soluble in it and there is no mutagenicity to the other four strains.

RESULTS

Soil Columns: TNT concentrations in leachates from soil columns were monitored over 100 days by HPLC. Initial TNT breakthrough occured at 14 days, and during the initial 35-day stabilization period concentrations of TNT in the leachates ranged up to 15 ppm (4.5 mg per 300 mL). Concentrations stabilized between 3 ppm and 7 ppm by day 36, when treatments began.

Levels of TNT in column A, the water control column, remained stable up to day 85 (2 to 4 ppm), and thereafter there was a gradual decline in concentration to about 1 ppm by day 95 and below 600 ppb by day 100 (Table 1).

TABLE 1. CONCENTRATION (PPM) OF TNT IN LEACHATES FROM THE SOIL COLUMNS.

	TREATMENT DAYS						
COLUMN	1 - 36[1]	36 - 45	46 - 55	56 - 65	66 - 75	76 - 85	86 - 95
1. WATER	—	2-3[2]	2-3	2-3	3-4	2-3	1-2
2. SODIUM HYDROXIDE	—	3-7	2-6	2-4	1-3	0.2-1	0.1-0.3
3. DUOQUAD T-50	—	3-6	2-5	1-6[3]	0.1-1	BD[4]-0.1	BD-0.05
4. DUOQUAD T-50 & SODIUM HYDROXIDE	—	3-5	1-3	0.4-2	BD[5]-0.4	BD	BD
MOLAR RATIO-DUOQUAD/TNT	—	0-4.5	4.5-8.9	10.1-20.2	21.3-31.4	32.5-42.7	43.8-53.9

1. TREATMENTS BEGAN DAY 36. DAYS 1-36 WERE WATER TREATMENTS FOR COLUMN EQUILIBRIUM.
2. CONCENTRATION (PPM)
3. DUOQUAD SATURATION ON DAY 65.
4. BD= BELOW DETECTION (<1PPB).
5. MOLAR RATIO = 30.3 ON DAY 75.

The leachate from column B, treated with sodium hydroxide, contained TNT at concentrations of 2 ppm to 7 ppm up to 65 days. The concentrations declined to about 1 ppm by day 73, below 300 ppb by day 87, and 100 ppb by day 100. The leachate from column C, the Duoquad T-50 treatment in distilled water, had levels of TNT above 1 ppm until day 64, below 1 ppm by day 66, below 100 ppb by day 75, and below 50 ppb by day 90. The leachate from column D, treated with Duoquad T-50 and sodium hydroxide, had levels of TNT below 2 ppm by day 60, below 200 ppb by day 68 and below detection limits (1 ppb) by day 75, when the molar ratio of Duoquad T-50 to TNT was 30.3 to 1 (Table 1).

By day 92, material in the leachates from columns C and D interferred with the HPLC analyses and further evaluation became difficult. The interferences were probably due to surfactant compounds in the leachate. The concentration of Duoquad T-50 in the influent and effluent from the two columns treated with surfactant was equivalent by day 65, indicating the columns were saturated with the surfactant.

Measurements of pH taken during the experiment were 6.1± 0.1, 7.0± 0.3 and 11.0± 0.5 for the influents to column A (water), column C (surfactant) and columns B and D (sodium hydroxide and surfactant with sodium hydroxide) (Table 2). Initially, all the effluents had a pH between 4.5 and 5.0. These readings changed during the ocurse of the experiment to near 7.0 for the water column leachate and near 8.0 for leachates from columns B and D. The pH of the effluent from column C remained near 4.5. The effluents from both water and surfactant columns were clear throughout the experiment while the effluents from columns B and D, treated with sodium hydroxide and surfactant with sodium hydroxide, turned brown, corresponding to the rise in pH (Figure 6).

TABLE 2. THE PH OF INFLUENTS AND EFFLUENTS FROM SOIL COLUMNS (PH ± 1 S. D.).

COLUMN	INFLUENT	INITIAL EFFLUENT	FINAL EFFLUENT	COLOR
A. WATER	6.1 ± 0.1[1]	5.0 ± 0.5	UNCHANGED	CLEAR
B. SODIUM HYDROXIDE	11.0 ± 0.5	5.0 ± 0.5	↑8[2]	DARK BROWN
C. DUOQUAD T-50	7.0 ± 0.3	4.5 ± 0.7	UNCHANGED	CLEAR
D. DUOQUAD T-50 & SODIUM HYDROXIDE	11.0 ± 0.5	5.0 ± 0.5	↑8	LIGHT BROWN

1. PH ± 1 S. D.
2. INCREASED TO PH 8.0 DURING THE EXPERIMENT.

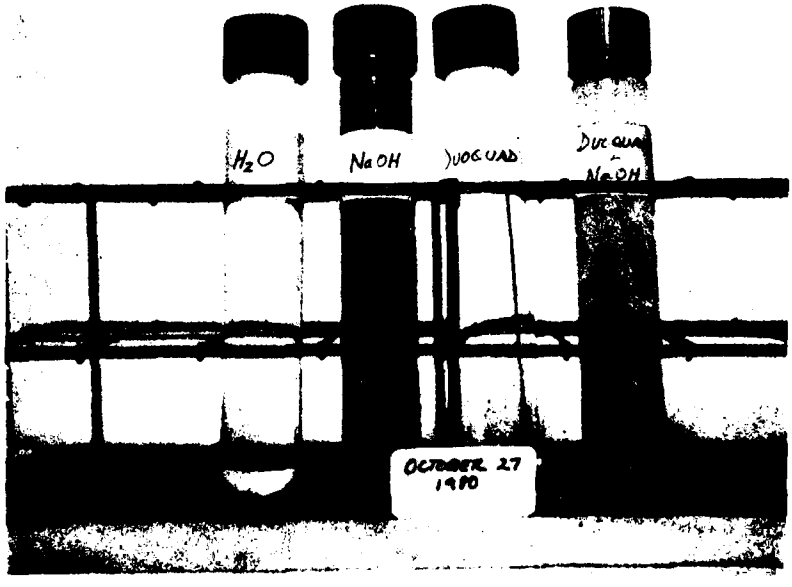

Figure 6. Coloration of leachates from the four soil columns contaminated with TNT.

Analysis of leachates from the soil columns by HPLC revealed two additional peaks eluting after TNT on the chromatograms. Peaks were identified by HPLC and GC/MS using standards. Retention times on the HPLC were identical for the 2A and 4A standards and the compounds isolated in the leachate extracts. The GC/MS analysis produced identical retention times in the GC analysis of the standards and unknowns. The 4A and 2A standards produced mass ions (m/z) of 52, 78, 104, 163, 180 and 197. The m/z 180 and m/z 197 are the most prominent ions while the ratios of mass ions (52/78/104) allows distinction between the 2A and 4A (4A ≈ 0.6/0.8/1.0, 2A ≈ 0.6/1.0/0.6). The unknows isolated from leachate samples revealed fragmentation patterns similar to the two standards, indicating both 2A and 4A were present in the column effluents.

The concentrations of both 2A and 4A were monitored in leachates from all four columns (Tables 3 and 4). The maximum concentrations after column equilibrium were 2 ppm and 3 ppm for 2A and 4A, respectively. Concentrations of both compounds gradually declined throughout the experiment, and by day 100, 200 ppb to 800 ppb concentrations were measured. Unlike the results with TNT, there was little difference in the concentrations of 2A and 4A in the effluents from the four columns throughout the experiment.

Standard Leaching Test: Traces of TNT, 2A and 4A were found in the water, sodium hydroxide and Duoquad T-50 treatment columns, while no traces of any of these three compounds were found in the Duoquad T-50/sodium hydroxide column. In all cases, concentrations were significantly reduced compared with the levels found during the experimental runs. Therefore, no measure of maximum concentration could be made.

Aqueous Biodegradation Studies: The distribution of recovered ^{14}C in the anaerobic biodegradation studies is summarized in Table 5. The radioactivity recovered in the extraction scheme accounted for 83.5% to 94.2% of the total.

The total $^{14}CO_2$ released during continuous trapping of effluent CO_2, combined with that released upon acidification of the media with HCl, was below 0.3% in the anaerobic flasks, and 3.7% and 4.5% in the aerobic flasks. There were no significant differences in $^{14}CO_2$ collected between the distilled water controls and the nutrient broth cultures. All pellets contained ^{14}C-labelled material, totalling 9.8% in the case of the Duomeen T-TNT complex, and progressively lesser amounts with the Arquad T-50-TNT and the Duoquad T-50-TNT complexes. Material retained on 0.2-μm filters contained significant amounts of radioactivity, up to 16.4% in the Duoquad T-50-TNT complex control and 4.2% with the other incubations.

The majority of the ^{14}C-labelled material was recovered in the two solvent washes of the incubation flasks after the aqueous media was removed. Almost 90% was recovered in these two fractions in the remaining incubations.

The aqueous fraction, after centrifugation and filtering still contained significant levels of radioactivity. The aqueous fraction from the Duoquad T-50-TNT complex contained higher levels of radioactivity than the Arquad or Duomeen complexes. The aqueous fraction was drawn through a SEP-PAK C-18 cartridge and the distribution of radioactivity is shown in Table 6. The majority of the ^{14}C-labelled material was retained on the cartridge and varying levels were eluted with methanol, leaving between 21% and 97% on the cartridge.

The methanol eluate from the cartridge and the solvent wash from the flask residues were analyzed for TNT and TNT reduction products by TLC, HPLC and

TABLE 3. CONCENTRATION (PPM) OF 2-AMINO-4,6-DINITROTOLUENE IN LEACHATES FROM THE SOIL COLUMNS.

	TREATMENT DAYS						
COLUMN	1 - 36[1]	36 - 45	46 - 55	56 - 65	66 - 75	76 - 85	86 - 95
1. WATER	—	0.3-2[2]	0.8-2	0.8-2	0.6-2	0.5-0.7	0.5-0.7
2. SODIUM HYDROXIDE	—	0.5-2	1-2	0.9-2	0.4-1	0.4-0.7	0.3-0.6
3. DUOQUAD T-50	—	0.4-2	1-2	0.9-2	0.8-2	0.5-1	0.4-0.8
4. DUOQUAD T-50 & SODIUM HYDROXIDE	—	0.4-2	0.9-2	0.7-2	0.3-1	0.3-0.7	0.2-0.5

1. TREATMENTS BEGAN DAY 36. DAYS 1-36 WERE WATER TREATMENTS FOR COLUMN EQUILIBRIUM.

2. CONCENTRATION (PPM)

TABLE 4. CONCENTRATION (PPM) OF 4-AMINO-2,6-DINITROTOLUENE IN LEACHATES FROM THE SOIL COLUMNS.

	TREATMENT DAYS						
COLUMN	1 - 36[1]	36 - 45	46 - 55	56 - 65	66 - 75	76 - 85	86 - 95
1. WATER	—	1-2[2]	1-2	1-2	0.7-2	0.5-0.9	0.5-0.8
2. SODIUM HYDROXIDE	—	1-2	1-2	1-2	0.4-1	0.3-0.7	0.3-0.6
3. DUOQUAD T-50	—	1-2	1-2	1-3	0.8-2	0.6-0.8	0.5-0.7
4. DUOQUAD T-50 & SODIUM HYDROXIDE	—	1-2	1-2	1-2	0.5-1	0.3-1	0.3-0.5

1. TREATMENTS BEGAN DAY 36. DAYS 1-36 WERE WATER TREATMENTS FOR COLUMN EQUILIBRIUM.

2. CONCENTRATION (PPM)

TABLE 5. DISTRIBUTION OF RADIOACTIVITY RECOVERED FROM THE AQUEOUS INCUBATIONS.

Treatment	Medium	N	Time (days)	RADIOACTIVITY (DISINTEGRATIONS PER MIN, PERCENT OF TOTAL)						
				$^{14}CO_2$	Pellet	Filter	Flask residue (A)[1]	Flask residue (B)[2]	Aqueous	Total
A. Anaerobic										
1. Duoquad-TNT complex	water	2	221	0.2	-[3]	16.4	58.7	5.9	11.4	92.6
2. Duomeen-TNT complex	water	2	221	0.1	-	0.1	81.8	8.3	3.9	94.2
3. Arquad-TNT complex	water	2	221	0.1	-	1.6	46.9	31.9	5.8	86.3
4. Duoquad-TNT complex	Nutrient broth	3	221	0.3	1.7	2.0	64.5	0.3	14.7	83.5
5. Duomeen-TNT complex	Nutrient broth	3	221	0.1	9.8	2.2	63.2	0.6	9.6	85.5
6. Arquad-TNT complex	Nutrient broth	3	221	0.1	6.4	1.1	51.4	22.2	8.8	90.0
B. Aerobic										
1. Duoquad-TNT complex	water	1	202	4.5	-	4.2	52.3	14.8	12.8	88.6
2. Duoquad-TNT complex	Nutrient broth	1	202	3.7	1.7	2.7	49.4	14.3	18.7	90.5

[1]Acetone or methylene chloride
[2]Dimethylsulfoxide
[3]No pellet present

TABLE 6. DISTRIBUTION OF RADIOACTIVITY IN THE AQUEOUS FRACTIONS.

TREATMENT	Medium	Radioactivity Recovered: Aqueous before extraction[1] (dpm)	Aqueous after extraction (dpm)	% removed	Total on column (dpm)	Methanol eluate (dpm)	% removed by Methanol	% retained on column
A. Anaerobic								
1. Duoquad-TNT complex	water	11440	1050	91	10390	8232	79	21
2. Duomeen-TNT complex	water	3937	420	89	3517	972	28	72
3. Arquad-TNT complex	water	5827	0	100	5827	2134	37	63
4. Duoquad-TNT complex	nutrient broth	14655	6090	59	8565	2470	29	71
5. Duomeen-TNT complex	nutrient broth	9624	0	100	9624	276	3	97
6. Arquad-TNT complex	nutrient broth	8784	2205	75	6579	750	11	89
B. Aerobic								
1. Duoquad-TNT complex	water	12810	0	100	12810	6572	51	49
2. Duoquad-TNT complex	nutrient broth	18690	2415	87	16275	5147	32	68

[1] Extraction by passage through C=18 SEP-PAK cartridge.

scintillation counting. No evidence was found for any of eight compounds (2A, 4A, TNT, 2,4DA, 2,6DA, 4OHA, 4,4'Az, and 2,2'Az). In general, ^{14}C-labelled material spotted for TLC remained at the origin (Figure 7).

Soil Biodegradation Studies: The distribution of recovered ^{14}C in the anaerobic soil biodegradation studies is summarized in Table 7. The radioactivity recovered in the extraction scheme accounted for 73.2% to 86.9% of the total.

Total $^{14}CO_2$ released during the incubations accounted for less than 0.8% of the total ^{14}C in the anaerobic incubations and 0.5% and 2.8% in the aerobic sterile control and test incubations. Flask residues were extracted with acetone or methylene chloride and then dimethylsulfoxide. Unlike the aqueous incubations, lower amounts of the ^{14}C-labelled material were recovered in these

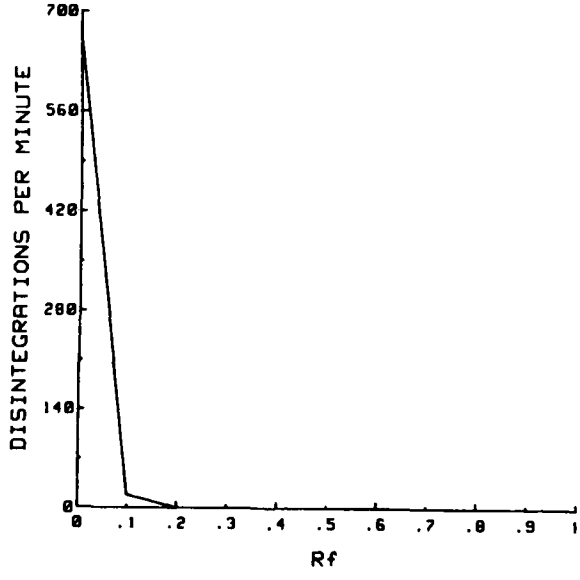

Figure 7. Radioactivity on the thin layer chromatograms from the flask residue solvent extract from the Duoquad T50-TNT complex developed in benzene/hexane/acetone.

TABLE 7. DISTRIBUTION OF RADIOACTIVITY RECOVERED FROM THE SOIL INCUBATIONS.

Treatment	Condition	N	Time (days)	RADIOACTIVITY (DISINTEGRATIONS PER MIN, PERCENT OF TOTAL)						
				$^{14}CO_2$	Flask residue (A)[1]	Flask residue (B)[2]	Soxhlet extraction (C)[3]	Soxhlet extraction (D)[4]	oxidation to $^{14}CO_2$	Total
A. Anaerobic										
1. Duoquad-TNT complex	sterile	2	223	0.3	5.7	3.4	15.9	0.1	54.2	79.6
2. Duomeen-TNT complex	sterile	2	223	0.2	9.3	7.0	6.8	20.1	39.7	83.1
3. Arquad-TNT complex	sterile	2	223	0.1	7.6	4.7	5.5	18.2	40.7	76.8
4. Duoquad-TNT complex	unsterile	3	223	0.8	8.2	4.3	17.7	0.5	49.0	80.5
5. Duomeen-TNT complex	unsterile	3	223	0.2	7.9	5.5	7.1	16.7	43.5	80.9
6. Arquad-TNT complex	unsterile	3	223	0.1	8.6	10.1	4.9	17.5	32.0	73.2
B. Aerobic										
1. Duoquad-TNT complex	sterile	1	209	0.5	9.2	12.7	17.3	0.1	44.7	84.5
2. Duoquad-TNT complex	unsterile	1	209	2.8	8.7	8.2	13.9	0.1	53.2	86.9

[1]Acetone or methylene chloride
[2]Dimethylsulfoxide
[3]Acetone
[4]Methylene Chloride

extracts; between 9.0% and 21.9%. The soil samples were Soxhlet extracted with acetone and methylene chloride, between 13.9% and 26.8% of the radioactivity was recovered, while the majority of the ^{14}C-labelled material was not solvent extractable. Upon incineration, between 39.7% and 54.2% of the total radioactivity was recovered from the soil samples.

The first flask residue solvent extract and the Soxhlet extraction samples were analyzed for TNT and TNT reduction products. As with the aqueous incubations, no evidence was found for any of these eight compounds as determined by TLC, HPLC, and scintillation counting.

The design of the original experiment included an examination of the differences between gas and steam sterilization and the different effects the sterilization techniques had on the complexes themselves in an nonsterile incubation. Figure 8 illustrates the differences in sterilization techniques using

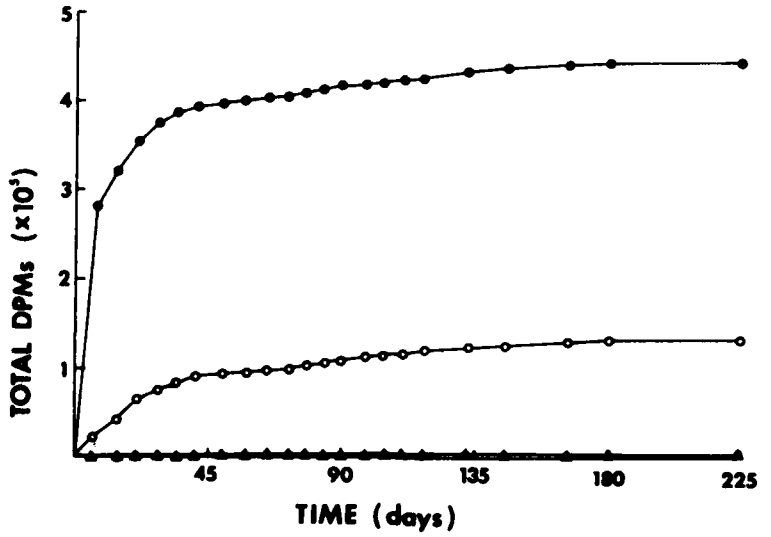

Figure 8. Recovery of $^{14}CO_2$ from ^{14}C-UL-sucrose in unsterile (●), gas sterilized (○) and steam sterilized (▲) soil.

^{14}C-labelled sucrose as a model control incubated under the same conditions as the complexes. The $^{14}CO_2$ recovery data indicated steam sterilization was a more efficient method than gas under the conditions of these experiments. The soil incubation with ^{14}C-UL-sucrose using steam sterilization accounted for 0.4% of the total radioactivity recovered in the unsterile control over 225 days, while the gas sterilized flask accounted for 30.2%. Despite these findings, in the experiments designed to evaluate the biodegradability of TNT-surfactant complexes in sterile incubations, there were little differences in the distribution of ^{14}C in the extracts. Similarly, little difference was found in the results from the extractions of the nonsterile flasks intended to discern differences in the complexes caused by gas and steam sterilization.

Mutagenicity Testing: Toxicity occurred at 5000 µg per plate with TNT and the Duoquad T-50-TNT complex, while the Duoquad T-50 surfactant produced toxicity at 500 µg per plate (Table 8). TNT was mutagenic to TA1538 at 50 and 500 µg per plate with and without metabolic activation; to TA98, TA100, and TA1537 at 500 µg per plate with activation; and to TA98 and TA100 without activation at 500 µg per plate. No evidence for mutagenicity was found for Duoquad T-50. The Duoquad T-50-TNT complex produced higher numbers of revertant colonies than TNT alone under the same test conditions and at the same concentrations. The complex also produced positive results for mutagenicity at lower concentrations than TNT. The complex tested positive in all five strains, at levels as low as 0.5 µg per plate with TA1538 and 5 µg per plate with TA98. At 500 µg per plate without activation there was a sixteen-fold increase in colony numbers formed by the complex compared with TNT alone. The dose response of TA1538 and TA98 without metabolic activation is illustrated in Figure 9.

TABLE 8. AMES TEST RESULTS FOR TNT, DUOQUAD T50 AND DUOQUAD T50-TNT COMPLEX.

AMES DATA — COMPOUNDS - 2,4,6-TRINITROTOLUENE, DUOQUAD T-50, TNT-DUOQUAD T-50 COMPLEX

	COMPOUND	Metabolic Activation	Micrograms Per Plate	ppm (approx)	HISTIDINE REVERTANTS PER PLATE ($\bar{X}$ ± 1 S.D.) TA1535	TA1537	TA1538	TA98	TA100
1.	Negative controls	-	0	0	8±2	18±4	11±7	25±9	128±26
		+	0	0	12±4	20±11	15±3	30±6	119±13
2.	Positive controls								
	Sodium azide	-	1	0.04	285±18				515±28
	9-aminoacridine	-	150	6		3875±489			
	4-nitrophenylenediamine	-	10	0.4			1527±176		
	2-nitrofluorene	-	50	2				1092±61	
	2-anthramine	+	2	0.08	143±51	229±42	1485±212	1477±148	1780±178
3.	2,4,6-Trinitrotoluene	-	5000	200	T[1]	T	T	T	T
		-	500	20	5±4	20±11	142±9*	338±112*	939±55*
		-	50	2	14±6	13±4	45±9*	48±8	301±72
		-	5	0.2	13±4	22±11	21±1	42±9	137±11
		+	5000	200	T	T	T	T	T
		+	500	20	23±1	126±17*	121±12*	420±85*	679±209*
		+	50	2	11±3	19±2	47±7*	32±14	203±17
		+	5	0.2	10±7	14±1	41±22	30±2	130±32
4.	Duoquad T-50	-	500	20	T	T	T	T	T
		-	100	4	18±5	41±12	20±7	31±2	103±10
		-	50	2	11±5	24±7	22±11	35±6	123±16
		-	5	0.2	18±7	20±11	13±5	31±5	140±31
		-	0.5	0.02	22±4	13±3	14±4	20±10	165±10
		+	500	20	T	T	T	T	T
		+	100	4	12±0	29±0	15±0	42±12	168±11
		+	50	2	11±2	21±6	18±2	49±12	125±11
		+	5	0.2	9±3	10±2	16±1	32±3	117±40
		+	0.5	0.02	21±2	32±7	18±2	18±12	182±27
5.	TNT-Duoquad T-50 Complex	-	5000	200	T	T	T	T	T
		-	500	20	42±13*	425±29*	2288±678*	2364±215*	965±51*
		-	50	2	15±6	75±12*	649±2*	605±215*	313±24
		-	5	0.2	17±2	29±7	104±6*	188±47*	182±25
		-	0.5	0.02	ND[2]	ND	33±3*	46±14	ND
		+	5000	200	T	240±22*	1629±148*	1344±31*	501±46*
		+	500	20	23±2	151±8*	824±65*	755±57*	583±13*
		+	50	2	8±3	27±11	116±10*	133±9*	165±2
		+	5	0.2	9±3	18±6	34±15	60±11	141±5

1 Toxic
2 No Data
* Mutagenic

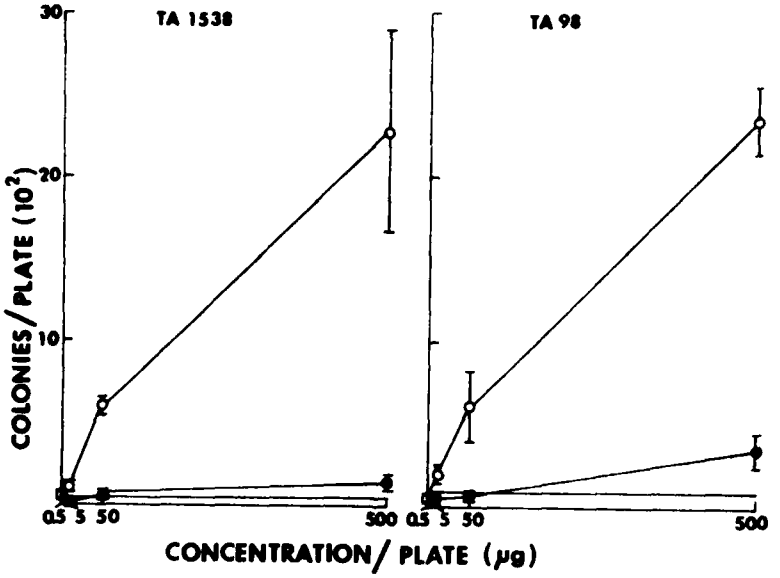

Figure 9. Dose responses of TA1538 and TA98 to TNT (●) and Duoquad T50-TNT complex (○) without activation.

The Arquad T-50-TNT complex also produced a higher mutation rate than TNT alone and demonstrated mutagenic effects at lower concentrations; 5 μg per plate with TA98, TA1537, and TA1538 (Table 9). Figure 10 illustrates the dose response of strains TA98 and TA1538 to TNT without activation. Toxicity due to the complex was evident at 500 μg per plate with both strains. The Arquad T-50 surfactant also tested positive for mutagenicity at 50 μg per plate with TA1538, with and without activation. Arquad T-50 was toxic at concentrations of 500 μg per plate.

The surfactant Duomeen T was not mutagenic. As with the other two TNT-surfactant complexes, the Duomeen T-TNT complex produced significantly greater numbers of revertant colonies than TNT alone, as well as demonstrated mutagenic

TABLE 9. AMES TEST RESULTS FOR TNT, ARQUAD T50 AND ARQUAD T50-TNT COMPLEX.

AMES DATA — COMPOUNDS - 2,4,6-TRINITROTOLUENE, ARQUAD T-50, TNT - ARQUAD T-50 COMPLEX

	COMPOUNDS	Metabolic Activation	Micrograms Per Plate	ppm (approx)	HISTIDINE REVERTANTS PER PLATE ($\bar{X} \pm 1$ S.D.) TA1535	TA1537	TA1538	TA98	TA100
1.	Negative controls	-	0	0	24±5	9±4	11±6	29±4	149±4
		+	0	0	13±4	8±3	11±6	20±5	113±3
2.	Positive controls								
	Sodium azide	-	1	0.04	418±56				490±57
	9-aminoacridine	-	150	6		2933±620			
	4-Nitrophenylenediamine	-	10	0.4			1139±84		
	2-nitrofluorene	-	50	2				884±206	
	2-anthramine	+	2	0.08	272±28	122±10	682±225	904±26	973±71
3.	2,4,6-Trinitrotoluene	-	5000	200	T[1]	T	T	T	T
		-	500	20	5±2	20±11	71±26*	184±61*	639±55*
		-	50	2	19±8	12±1	39±2*	50±10	187±13
		-	5	0.2	12±2	10±5	18±9	19±7	127±23
		-	0.5	0.02	10±3	9±3	7±4	45±5	ND[2]
		+	5000	200	T	T	T	T	T
		+	500	20	19±5	121±24*	150±17*	347±71*	522±76*
		+	50	2	20±5	5±2	20±5	51±14	140±18
		+	5	0.2	24±11	7±2	11±2	45±16	108±18
		+	0.5	0.02	7±3	14±1	10±2	45±5	93±17
4.	Arquad T-50	-	500	20	T	T	T	T	T
		-	50	2	6±8	T	33±10*	40±13	82±10
		-	5	0.2	22±21	10±5	24±4	29±7	87±12
		-	0.5	0.02	22±21	7±2	15±3	27±14	80±1
		-	0.05	0.002	18±11	9±2	16±4	29±12	103±24
		+	500	20	T	T	T	T	T
		+	50	2	7±2	4±4	33±13*	37±11	86±9
		+	5	0.2	10±2	5±3	14±4	37±4	89±21
		+	0.5	0.02	21±4	8±6	22±10	34±12	140±20
		+	0.05	0.002	10±0	9±3	27±9	21±11	113±16
5.	TNT-Arquad T-50 Complex	-	5000	200	T	T	T	T	T
		-	500	20	33±2	224±91*	395±139*	573±123*	163±9
		-	50	2	66±39	98±8*	971±153*	781±55*	240±14
		-	5	0.2	30±6	65±8*	154±19*	108±15*	145±8
		-	0.5	0.02	7±4	19±8	13±3	49±10	141±43
		+	5000	200	T	T	T	T	T
		+	500	20	20±2	163±20*	1550±165*	777±66*	250±13
		+	50	2	20±3	38±3*	926±746*	194±33*	201±6
		+	5	0.2	16±2	25±2*	1170±253*	53±14	127±13
		+	0.5	0.02	13±10	15±6	13±2	50±5	102±6

[1] Toxic

[2] No Data

* Mutagenic

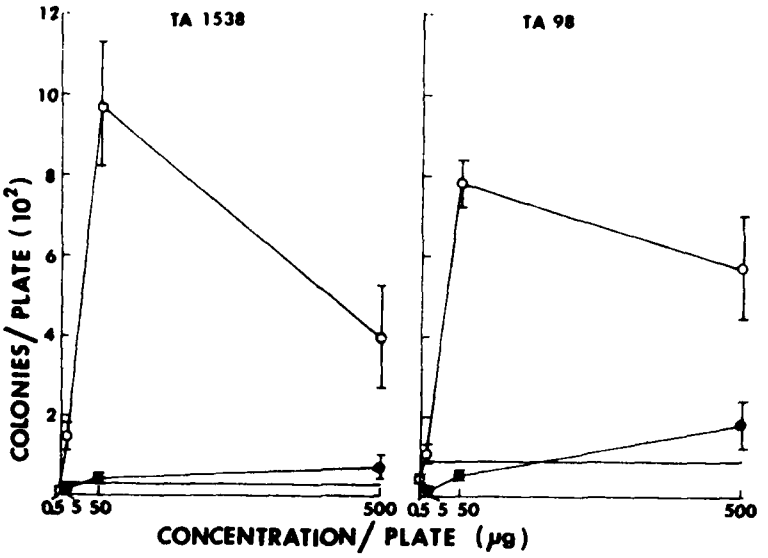

Figure 10. Dose response of TA1538 and TA98 to TNT (●) and Arquad T50-TNT complex (O) without activation.

potential at concentrations down to 5 μg per plate with TA1538 (Table 10, Figure 11). The mutagenic effect of methylene chloride on TA98 is indicated in Table 10.

Table 11 and Figure 12 present the responses of the five strains to the dilution series of the Composition B filtrate. At or below the process concentration no mutagenic activity was evident, but upon concentration positive results were found with TA98, TA1535, TA1537 and TA1538.

DISCUSSION

The Ames test results indicate a significant hazard associated with the TNT-surfactant complexes. There was an increase in mutation rate caused by the complexes when compared with the rate resulting from exposure to TNT or the

TABLE 10. AMES TEST RESULTS FOR TNT, DUOMEEN T AND DUOMEEN T-TNT COMPLEX.

AMES DATA — COMPOUNDS - 2,4,6-TRINITROTOLUENE, DUOMEEN T, TNT-DUOMEEN T COMPLEX, METHYLENE CHLORIDE

	COMPOUND	Metabolic Activation	Micrograms Per Plate	ppm (approx)	HISTIDINE REVERTANTS PER PLATE ($\bar{X} \pm 1$ S.D.) TA1535	TA1537	TA1538	TA98	TA100
1.	Negative controls	-	0	0	11±6	6±1	12±2	18±3	170±58
		+	0	0	9±3	12±1	13±3	22±6	148±14
2.	Positive controls								
	Sodium azide	-	1	0.04	167±42				391±40
	9-aminoacridine	-	150	6		2147±401			
	4-nitrophenylenediamine	-	10	0.4			1113±89		
	2-nitrofluorene	-	50	2				1534±96	
	2-anthramine	+	2	0.08	143±41	195±8	1760±43	1215±237	1885±155
3.	Methylene chloride	-	(50 µl)		13±7	12±5	13±5	425±53*	285±81
		+	(50 µl)		10±4	5±2	17±2	266±66*	193±35
4.	Duomeen T	-	50	2	T[1]	T	T		T
	(Methylene chloride)	-	5	0.2	5±1	3±2	7±2		T
		-	0.5	0.02	10±3	4±3	6±2		168±64
		-	0.05	0.002	11±3	ND[2]	9±1		167±26
		+	50	2	T	T	T		T
		+	5	0.2	9±2	5±2	11±5		146±19
		+	0.5	0.02	9±1	10±5	12±4		244±25
		+	0.05	0.002	9±4	7±5	18±8		253±60
5.	2,4,6-Trinitrotoluene	-	5000	200	T	T	T		T
	(Methylene chloride)	-	500	20	6±4	32±15	67±21*		T
		-	50	2	17±3	7±3	55±13*		258±45
		-	5	0.2	8±4	6±1	17±7		203±62
		+	5000	200	T	T	T		T
		+	500	20	11±6	39±8*	69±11*		433±93
		+	50	2	10±2	8±4	21±3		250±42
		+	5	0.2	11±2	11±5	24±13		281±47
6.	TNT-Duomeen T	-	5000	200	10±8	103±1*	401±38*		224±22
	complex	-	500	20	26±5	54±7*	223±7*		186±24
	(Methylene chloride)	-	50	2	13±5	11±4	85±10*		150±29
		-	5	0.2	14±2	9±2	7±2		176±50
		+	5000	200	13±9	38±12*	342±13*		209±89
		+	500	20	13±1	22±13*	150±65*		256±59
		+	50	2	12±3	8±3	53±15*		266±50
		+	5	0.2	14±4	10±3	54±15*		286±17

1 Toxic
2 No Data
* Mutagenic

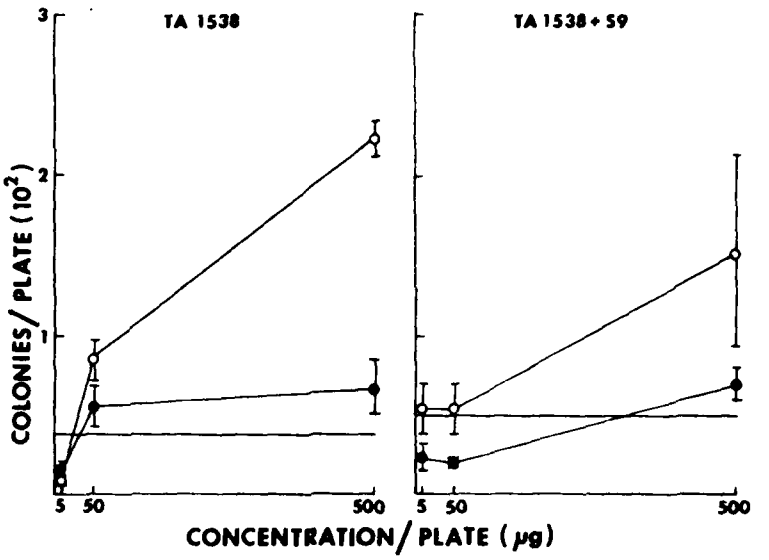

Figure 11. Dose response of TA1538 with (S9) and without activation to TNT (●) and Duomeen T-TNT complex (O).

TABLE 11. AMES TEST RESULTS FOR THE FILTRATE AFTER PROCESS TREATMENT.

FILTRATE - DUOQUAD T-50 TNT Treatment

	COMPOUND	Metabolic Activation	Concentration	Microliters Per Plate	HISTIDINE REVERTANTS PER PLATE ($\bar{X} \pm 1$ S.D.) TA1535	TA1537	TA1538	TA98	TA100
1.	Filtrate	-	100X	250	25±5*	72±7*	311±46*	314±18*	322±25
		-	10X	250	12±4	38±2	76±24*	76±12*	226±38
		-	Normal	250	17±5	30±12	16±10	31±6	106±4
		-	1/10	100	15±5	25±12	16±4	23±8	163±8
		-	1/100	100	12±3	36±8	23±3	32±3	155±13
		+	100X	250	43±2*	32±6	174±38*	167±14*	260±14
		+	10X	250	14±4	15±9	33±6	54±9	180±34
		+	Normal	250	12±3	14±11	21±2	51±8	192±3
		+	1/10	100	15±1	24±2	22±5	31±2	149±5
		+	1/100	100	17±8	23±7	24±5	37±13	186±26

* Mutagenic

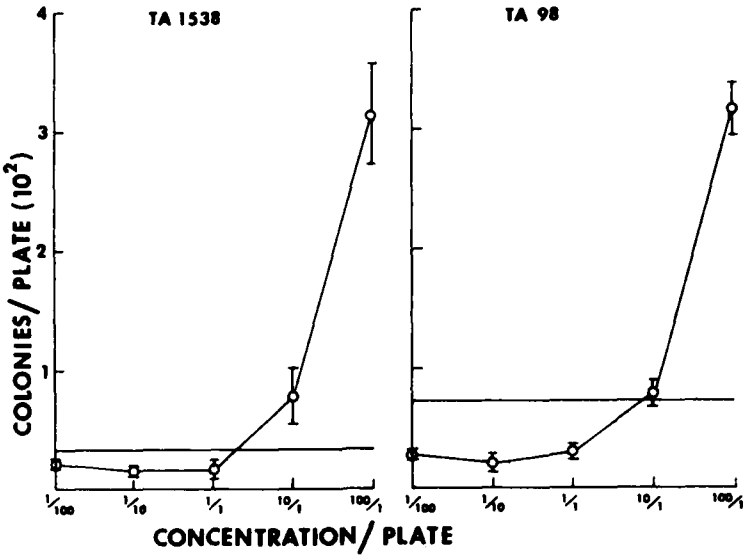

Figure 12. Dose response of TA1538 and TA98 to different concentrations of Comp. B filtrate after treatment with Duoquad T50 at pH 11.0 and filtration.

surfactants alone under the same experimental conditions. This effect occurs with all three complexes (Duoquad T-50-, Duomeen T- and Arquad T-50-TNT). The concentrations at which positive findings were detected were below the levels for TNT alone, as low as 0.5 µg per plate with the Duoquad-T50-TNT complex. The surfactants, Duoquad T-50 and Duomeen T, tested negative, but Arquad T-50 gave a positive result with TA1538 at 50 µg per plate. This may be due to the surfactant itself or impurities arising during its manufacture.

The surfactants were toxic to the *Salmonella* strains at concentrations as low as 50 g per plate with Duomeen T and 500 g per plate with Arquad T-50 and Duoquad T-50. This reflects the biocidal nature of these compounds, which limits the ability to test for mutagenicity at higher concentrations.

The filtrate from a Composition B solution, after treatment with Duoquad T-50 at pH 11, also demonstrated mutagenic potential when concentrated. This illustrates the need for further investigation into the chemical moieties present in the waste stream after treatment and filtration. The mutagenic agents could be residual TNT or TNT-surfactant complex, hydrolysis products from the RDX present in the Composition B waste water, or other compounds formed during this reaction. An answer to this problem should be determined before a final assessment can be made concerning the quality of the effluent from this treatment process. If this process is used for on-line treatment, considerable fluctations would be expected in feed composition, and maximum efficiency of reaction would not always be possible. Therefore, although some concentration of filtrate was required for detectable mutagenicity, there nevertheless exists the potential for problems to arise during less efficient periods of operation.

The soil lysimeter studies revealed a number of problems with *in situ* treatment of TNT-contaminated soil. The complexing of TNT, was very inefficient when compared to treatment of TNT-contaminated water. A molar ratio of 30.3 to 1 of Duoquad T-50 surfactant to TNT had been reached at the point the TNT concentration in the effluent, from the column treated with Duoquad T-50 and sodium hydroxide, fell below detection limits (1 ppb). The molar ratios were 24.7 to 1 and 14.6 to 1 when the concentration of TNT in the leachate fell below 100 ppb and 1 ppm, respectively. This can be compared with aqueous systems where a 0.3 to 1 molar ratio is required to reduce TNT concentrations below 1 ppm. It is clear that much higher concentrations of surfactants would be required to treat TNT-contaminated soils and the corresponding costs would also escalate.

The soil columns became saturated with surfactant (day 65) before TNT levels dropped below detection limits (day 73). Once this saturation point was reached, equivalent amounts of surfactant were found in the leachate and the influent on each treatment day. Significant levels were also leaching out of the columns prior to saturation. Groundwater contamination problems would be compounded by the leaching of this excess surfactant.

There were also problems caused by the excess sodium hydroxide required with the surfactant treatment. As the pH of the effluents changed during the experiment, a corresponding change occurred in the coloration of the effluent. The columns treated with sodium hydroxide or with sodium hydroxide plus surfactant, produced effluents which changed from clear to brown. At the same time, the pH of these effluents rose from 5.0 to near 8.0. This coloration is due to the leaching of soil organic matter components (humic acids, fulvic acids) from the soil.[30] These components are essential to soil, providing for soil quality and tilth, cation exchange capacity, a store of nitrogen and a source of plant hormones. The application of excess sodium hydroxide to any soil in an effort to immobilize TNT diminishes the basic soil quality. Effluents from the columns not treated with alkali remained clear throughout the experiment.

Two TNT reduction products (2A, 4A) were detected in the effluents from all four columns. The alkaline surfactant treatment did not result in significantly lower concentrations of these two compounds. Since these transformation products represent environmental hazards, a treatment to complex TNT should

[30]F. J. Stevenson. 1965. In Methods of Soil Analysis Part 2. 1409-1421. C. A. Black, Ed. Am. Soc. Agronomy, Madison, WI.

also treat TNT metabolites which have formed in the contaminated soil. This was not the case for the amino reduction products and significant soil and groundwater contamination problems would remain. No other TNT metabolic reduction products were detected in the column leachates.

The results from the aqueous biodegradation studies indicate that the complexes are not stable in water for a long period. In the aqueous incubations the majority of the complex appears to have remained in the water insoluble form which was extractable as the two solvent extracts. However, over the seven-month incubation period, significant quantities of radioactivity were found in the water soluble fraction. The formation of these water soluble materials was independent of microbial activity. No free TNT or TNT reduction products were detected in this water soluble fraction, nor in any of the extracts analyzed from either the aqueous or soil incubations.

The structures of the compounds produced by long-term exposure of the complex to water are not yet known. However, their polarities are such that they are partially soluble or in suspension in water but become bound to the C-18 SEP-PAK cartridges. These products may also have been formed in the soil, but probably became bound to the soil organic matter and unextractable with organic solvents. Even if these products were slowly released from the complex they would very likely become bound to the soil and unavailable for groundwater contamination until the system became saturated.

In both the soil and water studies, there were little differences between the results of the sterile and nonsterile incubations. Therefore, the changes observed during the long-term incubations are attributable to nonbiological factors and not microbial susceptibility of the complex. All three of the

complexes gave patterns of distribution of the ^{14}C-labelled material which were similar.

The Ames screening test for mutagenicity revealed that these complexes represent a significant mutagenic hazard. Landfilling and incineration of these complexes are potential final disposal alternatives. The choice will in part be determined by the long-term stability of the complexes in soil and water. Since concern is now raised regarding the mutagenic potential and long-term stability of these compounds, it is questionable whether their use as landfill is a viable alternative.

CONCLUSIONS

A number of problem areas have been raised regarding the use of amino surfactants at a high pH to treat TNT-contaminated soil and water. The complexes produced higher mutation rates than TNT in Ames testing. Tests with soil columns contaminated with TNT indicated (1) a high molar ratio of surfactant to TNT is required for complexing, (2) leachate problems are caused by the excess surfactant and sodium hydroxide needed to complex the TNT and (3) an inability of the treatment to complex TNT reduction metabolites, 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene. Soil and water biodegradation studies indicated possible problems with long-term stability of these complexes due to nonbiological factors. More work is needed to fully assess the quality of the water filtrate from an actual munitions waste treatment process after complexing and filtration, in order to identify the residual compounds which pose a hazard. Incineration may be preferred to landfilling for the final disposal of these complexes because of the problems raised in these studies. The proposed immobilization of TNT in TNT-contaminated soils using amino surfactants at high pH can not be recommended as a decontamination treatment in its present concept because of the adverse factors uncovered in these studies.

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